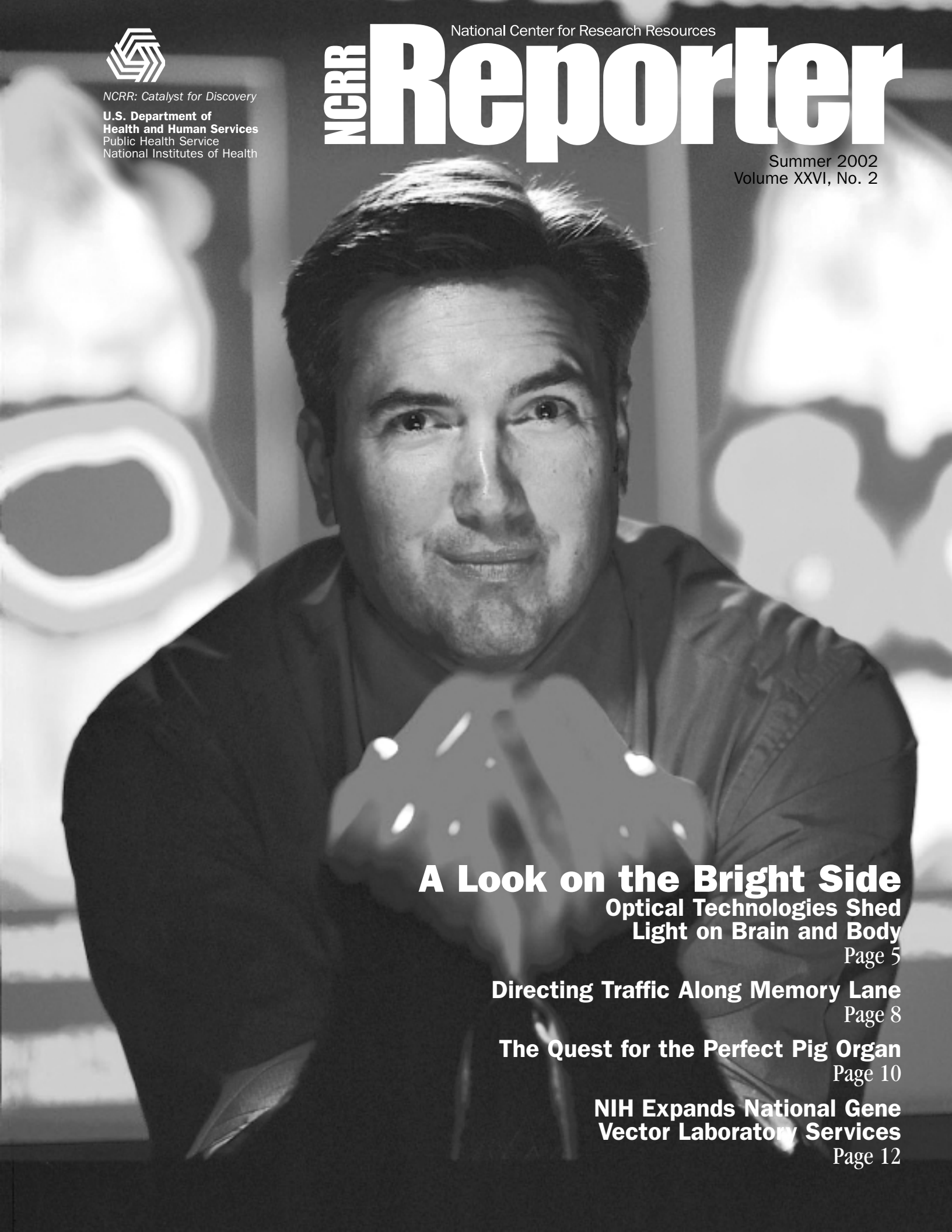
National Center for Research Resources
NCRR Reporter
Summer 2002
Volume XXVI, No. 2

NCRR: Catalyst for Discovery

U.S. Department of Health and Human Services
Public Health Service
National Institutes of Health



A Look on the Bright Side

From the Director

As clinical researchers address more complex queries, an array of cutting-edge resources is essential to further their investigations. To address these needs, the National Center for Research Resources (NCRR), acting in its role as the nation's leading supplier of biomedical research resources, continues to expand its support to clinical investigators. NCRR is providing access to specialized environments, sophisticated technologies, data networks, and other tailored resources that help advance patient-oriented research.

The NCRR-supported network of more than 80 General Clinical Research Centers (GCRCs) has long provided the specialized environments essential to facilitating countless investigations of human diseases, both rare and common. For example, the cover story for this issue highlights the work of Stanford University investigators who relied on the GCRC infrastructure to bring promising new techniques in biomedical optics from the bench to the patient. (See "A Look on the Bright Side," page 5.)

Sophisticated core genotyping resources are bringing advanced technologies to clinical investigators nationwide. For instance, the genomics laboratories at the GCRC at San Francisco General Hospital not only allow investigators to identify specific disease-related genes and unique therapeutic interventions, but they also offer an environment for training young investigators in the analysis of microarray data. Other specialized laboratories and facilities across the country support cell sorting and pharmacogenetics.

NCRR recently established specialized resources to optimize therapies to eliminate or possibly cure type 1 diabetes. Researchers at the NCRR-supported Islet Cell Resources will use advanced technologies to harvest, isolate, and distribute insulin-producing cells for transplantation into patients with type 1 diabetes. Likewise, another network of specialized resources, the National Gene Vector Laboratories (NGVLs), help researchers to obtain adequate quantities of clinical-grade vectors for human gene transfer. (See "NIH Expands National Gene Vector Laboratory Services," page 12.)

Data networks also facilitate clinical research. For instance, the Biomedical Informatics Research Network (BIRN)—a collaboration of the San Diego Supercomputer Center, the National Science Foundation, several universities, and NCRR—aims to bring together groups of investigators with complementary expertise to collaborate in a neuroscience test bed. Subsequent test beds will target other promising research areas, such as an international focus on computational cell biology. A key feature of these collective efforts is to create infrastructure that can be deployed rapidly and broadly.

These are just a few instances where specialized environments, sophisticated technologies, and advanced data networks are benefiting patient-oriented research. Multidisciplinary teams of physician-investigators, physicists, bioinformaticists, physical chemists, structural biologists, and others will continue to revolutionize biomedical research, and NCRR will continue to help lead the revolution by providing dedicated resources.

Judith L. Vaitukaitis, M.D.
Director, NCRR

NCRR Reporter

www.ncrr.nih.gov

This quarterly publication of the National Center for Research Resources fosters communication, collaboration, and resource sharing in areas of current interest to scientists and the public.

Editor:
Joyce McDonald, NCRR

Design:
Medical Arts and Photography Branch, NIH

Published for NCRR by the Research Resources Information Center (SSN: 0160-807X)

RRIC Director:
Victoria L. Contie

Senior Science Writer:
Steven Stocker

Editorial Assistant:
Shirley Coney-Johnson

Please send comments and ideas about future articles to:
Office of Science Policy
and Public Liaison, NCRR/NIH
6705 Rockledge Drive
Bethesda, MD 20892-7965
Telephone: (301) 435-0888
Fax: (301) 480-3558
E-mail: info@ncrr.nih.gov

Cover: Dr. Christopher Contag demonstrates that ordinary light can be transmitted through body tissues—in this case, the tissues of his hands. In the background are examples of in vivo bioluminescent imaging, in which light emitted by glowing bacteria is detected in the organs of living mice by a camera outside the body. (Photo by Steve Fisch)

NCRR Reports

Research findings with NCRR support

Obese Children at Risk for Diabetes

Blame it on eating too much pizza while sitting in front of too much television. According to studies conducted over the past 20 years, kids around the world are growing increasingly obese and, probably not coincidentally, are developing type 2 diabetes at alarming rates.

To the epidemiological evidence showing a link between pre-adult obesity and type 2 diabetes, researchers at Yale University School of Medicine in New Haven, Connecticut, have now added metabolic evidence. In a cohort of 167 obese children and adolescents who were evaluated in NCRR-funded General Clinical Research Centers, nearly 25 percent had impaired glucose tolerance, according to the oral glucose tolerance test (OGTT). In this test, a person drinks a glucose syrup and the blood sugar is measured as plasma glucose two hours later. An abnormally high level generally indicates reduced insulin secretion or effectiveness—the hallmark of diabetes.

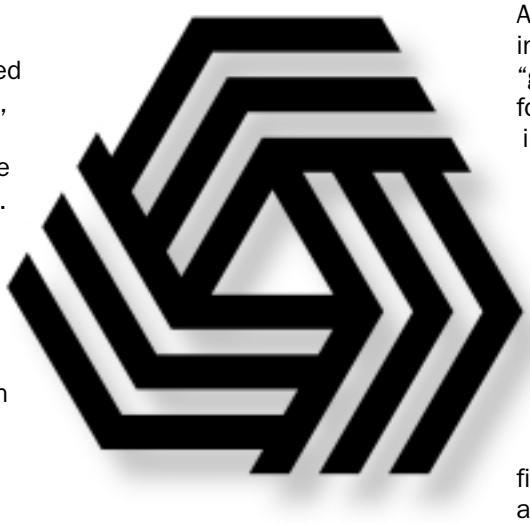
The results suggest that the OGTT might be used to identify obese children at high risk for diabetes in order to target them for intensive weight-loss treatment.

—*New England Journal of Medicine* 346:802-810, 2002.

The Double-Barreled Chloride Channel

Besides being one-half of the salt molecule that graces our french fries, the chloride ion also has important roles to play in the body, such as regulating the contraction of skeletal muscles and the secretion of other ions.

These tasks generally involve the passive flow of chloride ions through selective channels in cell membranes. Using NCRR-funded synchrotron and mass spectrometry resources, scientists at Rockefeller University have determined the atomic structures of two bacterial chloride channels, marking the first time that anyone has deciphered the structure of a negative-ion channel. Previously, the same group identified the structure of a bacterial channel for potassium, a positive ion.



Unlike the potassium channel, which consists of a single pore surrounded by four protein subunits, the chloride channel consists of two protein subunits, each with a central pore. As seen in a cross-section of the membrane, each chloride channel pore is shaped like an hourglass—wide at both ends and narrow in the middle. The researchers suggest that this middle section is where the flow of chloride ions through the channel is regulated. The findings may aid development of new therapies for diseases caused by chloride channel abnormalities.

—*Nature* 415:287-294, 2002.

Keeping Neurons in Their Place

Studies of early nervous system development often focus on how the neurons in embryos form connections with each other. Little, if any, attention has been paid to what maintains these connections once they are formed.

Now scientists at Columbia University and the NCRR-funded Center for *C. elegans* Anatomy at Albert Einstein College of Medicine in New York City have identified six "glue" genes that appear to be crucial for maintaining neuronal connections in the roundworm *Caenorhabditis elegans*. In particular, these genes encode proteins that keep axons—the long fibers that extend from neurons and transmit impulses to other cells—in their correct locations. The researchers first noticed that the genes, which they called zig genes, were expressed in a ventral nerve cord (VNC) neuron called PVT during the first larval stage, which is after most axons in the worm have completed their outgrowth. When the scientists destroyed PVT at this stage with a laser beam, the VNC axons in about a third of the worms seemed to become disoriented and wandered over to the opposite side of the cord. Similar results occurred in worm mutants lacking one of the six genes, called *zig-4*. The researchers speculate that *zig*-like genes also may operate after embryonic development in higher animals to keep their nervous systems from becoming unraveled.

—*Science* 295:686-690, 2002.

S.S.

A Look on the Bright Side

Optical Technologies Shed Light on Brain and Body

by Addison Greenwood

Scientists at Stanford University have taken a shine to the newborn brain—literally. For more than a decade, Drs. David K. Stevenson, David Benaron, and their colleagues have been exploring the potential of light to monitor areas of brain activity in critically ill infants. By shining light into the skull, researchers can gain insight into brain functions or injuries based on how the light diffuses through brain tissues. In studies led by Dr. Christopher Contag, Stanford scientists have also turned the technique inside-out, so to speak, by tagging cells inside the body with genes that produce glowing proteins and then tracking the light that emerges.

Such innovative light-based technologies—known collectively as biomedical optics—have many potential applications, including real-time diagnosis and evaluation of brain injury, sepsis, and cancer progression. As the scientists have moved their light-based investigations from in vitro and animal models to clinical studies over the past 10 years, they have relied on the resources of the NCRR-supported General Clinical Research Center (GCRC) at Stanford University in Stanford, California.

Nowhere is the power of biomedical optics more needed than with critically ill newborns, says Dr. Stevenson, an associate program director of the GCRC and the Harold K. Faber Professor of Pediatrics. Clinicians have become reliant on images that show functional activities, but the imaging techniques that produce those images are problematic with premature infants. PET (positron emission tomography) and SPECT (single-photon emission computed tomography), for instance, are not available in all facilities and require moving the critically ill infants to a radiology suite. And although MRI technology has become the workhorse of clinical functional imaging, patients must remain motionless while MRI data are being collected. "When a crisis develops in a critically ill newborn, it's not so easy to ship a baby down to radiology and instruct that baby to hold still for 30 minutes to an hour inside a noisy MRI machine," says Dr. Stevenson. Thus the ideal imaging modality for neonates would be fast, portable, and noninvasive. With critically ill patients of any age, clinicians also would benefit from a continuous imaging system that could quickly detect and, where possible, respond to changes in clinical states.

Enter Drs. Benaron, Stevenson, and their colleague Dr. Susan Hintz, with a relatively new imaging technology known as DOTS (diffuse optical tomography system). Developed by Dr. David Boas and other scientists at the Massachusetts General Hospital's Nuclear Magnetic Resonance (NMR) Center in Boston, DOTS was designed to deliver functional images of the brain. The procedure was further refined, in coordination with the Stanford research team, to enable analysis of the brains of tiny premature infants. The process employs a baby-friendly, soft, and flexible cap. Built into the cap are two grids of optical probes: a 3x3 set of laser light diodes, which sends light into the brain, and a 4x4 set of silicon photodiode detectors. Clinicians use a laptop computer to control how light is

To obtain images of brain blood flow, Dr. Susan Hintz attaches a cap with optical probes to the head of an infant. *(Photo by Dr. David Boas of the NMR Center, Massachusetts General Hospital. Dr. Boas helped to develop the DOTS device and modify it for use with infants.)*

transmitted from, and collected by, these optical probes.

Most optical imaging systems are spectroscopic—that is, they take advantage of the fact that distinct wavelengths of light react differentially with various tissues and molecules. For instance, when a flashlight is placed against the hand, the light entering the body is white, but what emerges appears red; the shorter wavelengths of blue and green light have been absorbed by the tissues of the hand. "Every wavelength represents a new contrast agent," and thus reveals distinct information, says Dr. Benaron. The clinician can modify the wavelength to search for specific targets, such as hemoglobin, bilirubin, or even the fat and water content of tissues. In this case, DOTS employs red and near-infrared light to home in on the oxygen transported by hemoglobin as it fuels brain activity.

The ability of optical imaging to discriminate changes in the volume of hemoglobin in local regions of the brain is impressive.

Using harmless near-infrared light, Dr. David Stevenson and his colleagues can detect brain blood flow, and thus brain activity, in premature infants. *(Photo by Steve Fisch)*

The ability of optical imaging to discriminate changes in the volume of hemoglobin in local regions of the brain is impressive for a bedside, noninvasive device. Advantages are its near-real-time results; its safety, which allows continuous use; its portablilty; and its relatively low cost. But as with all optical approaches, its resolution and accuracy fall off sharply as the imaging goes deeper into the body.

Optical systems like DOTS, which are based on diffusion theory, show tremendous promise in capturing functional images within about 5 cm of the body's surface. "At these depths, optical imaging can be 1,000 times as sensitive as currently developed nuclear medicine applications of PET or SPECT," says Dr. Benaron. But deeper into the body's tissues, the physics of photon scattering and absorption becomes more problematic and difficult to compute. For whole-body or deep-tissue imaging, optical systems tend to be inferior to other imaging techniques in both resolution and detectability.

An altogether different approach to using light in biomedical imaging was also developed by the biomedical optics group at Stanford, where

Dr. Christopher Contag is an assistant professor in the departments of pediatrics, radiology, and microbiology and immunology. It occurred to him to turn the problem inside out. What if, he wondered, you could label a specific cell type or biological process with its own source of light? "Since mammalian tissues don't produce much internal light, the signal-to-noise ratios would be extraordinary," says Dr. Contag. And if light could make its way through the intervening tissues to be detected at the surface of the body, "the sensitivity of detecting biological changes would be fantastic," he says.

Dr. Contag, together with wife Dr. Pamela Contag and Dr. Benaron, decided to employ the light-producing enzyme luciferase—similar to the enzyme that gives fireflies their glow—in a novel optical technique called in vivo bioluminescent imaging (BLI). In pioneering experiments conducted in the mid-1990s, the scientists tagged pathogenic bacteria with the luciferase gene, creating microbes that glowed like "little light bulbs," says Dr. Christopher Contag. When these bacteria were sandwiched within layers of living tissues, their glow could be detected on the tissues' surface. "Pretty soon we were tracking glow-germs in living mice as the bacteria adhered to and invaded the cells of infected animals," he says. By following the glow of tagged salmonella as they moved through infected animals, the researchers discovered that the bacteria establish a reservoir of colonies at the junction between the large and small intestines, in a region known as the cecum.

Watching the movement of bioluminescent organisms proved to be only the first step. Dr. Contag and many other investigators have demonstrated that BLI has a broad range of applications. By attaching the luciferase gene to on-off switches called promoters—from viruses or their mammalian hosts—Dr. Contag's research team bioengineered "indicator lights" to signal when a gene turns on or off. This technique promises to be especially useful for DNA-based therapeutics, which require effective monitoring of gene transfer and expression at various tissue sites. "We've developed BLI into a powerful tool that enables study of infection, gene expression, tumor growth, and protein function in living animal models of human biology and disease," says Dr. Christopher Contag.

The scientists tagged pathogenic bacteria with the luciferase gene, creating microbes that glowed like "little light bulbs."

The list of promising applications of biomedical optics is long and diverse. Light-based techniques for detecting and typing tumors—especially in breast, prostate, and cervical cancers—are now being evaluated at several clinical sites, and optical techniques may soon help clinicians to evaluate stroke and other brain injuries, detect physiologic changes, and reveal patterns of gene expression in living animals.

"Clinical researchers are no longer content with a snapshot of anatomy, a static X-ray, or a histologist's report of dead tissue that has been removed from its biological context," comments Dr. Benaron. "Imaging as we have come to know it is undergoing a profound revolution."

This research is supported in part by the Division of Clinical Research of the National Center for Research Resources, the National Institute of Neurological Disorders and Stroke, the United Cerebral Palsy Foundation, and the Office of Naval Research.

For more information about the NCRR Division of Clinical Research, see www.ncrr.nih.gov/clinical_rsrch.asp.

Additional Reading

1. Hintz S. R., Benaron, D. A., Siegel, A. M., et al., Bedside functional imaging of the premature infant brain during passive motor activiation. *Journal of Perinatal Medicine* 29:335-343, 2001.
2. Benaron, D. C., Hintz, S. R., Villringer, A., et al., Noninvasive functional imaging of human brain using light. *Journal of Cerebral Blood Flow and Metabolism* 20:469-477, 2000.
3. Contag, P R., Olomu, I. N., Stevenson, D. K., and Contag, C. H., Bioluminescent indicators in living mammals. *Nature Medicine* 4:245-247, 1998.

Research Highlights

Directing Traffic Along Memory Lane

When I was younger I could remember anything, whether it had happened or not; but my faculties are decaying now, and soon I shall be so I cannot remember any but the things that never happened.
—Mark Twain

Mark Twain is not alone in being vexed by memories. Most people experience deterioration in memory as they age, whereas others may suffer from memories that are alarmingly vivid. People with post-traumatic stress disorder, for instance, are repeatedly reminded of traumatic events through flashbacks and nightmares. By uncovering the molecular underpinnings of memory, scientists hope to develop new strategies for treating these and other memory-related disorders.

Since the 1960s, researchers have known that long-term memory formation involved protein synthesis in the brain. However, exactly which proteins were created and how they worked largely remained a mystery. Scientists have long sought to identify these proteins, and possibly develop drugs that interact with them, thereby influencing the establishment and retention of memory.

Dr. Alcino Silva (right) and Dr. Sheena Josselyn study the relationships between gene expression and memory in mice.
(Photo by Tawnie Silva, University of California, Los Angeles)

Now scientists supported in part by the NCRR Division of Research Infrastructure (DRI) provide evidence that a protein called cAMP-responsive element-binding protein, or CREB, has a dual role in the formation of long-term memories: Soon after an event occurs, CREB binds to DNA and activates genes that help form a transitory, unstable version of the memory; later, when that memory is recalled for the first time, CREB turns on genes that help promote the storage of a stable version of that memory in the brain.

To determine CREB's role in learning and memory, Dr. Alcino J. Silva, professor of neurobiology, psychiatry, and psychology at the University of California, Los Angeles, and Dr. Sandra Peña de Ortiz, associate professor of biology at the University of Puerto Rico, Rio Piedras, worked with their colleagues to block CREB function in a type of learning called fear conditioning. In these experiments, mice were placed in a chamber where they were given footshocks. Memory was assessed by later putting the mice back in the same chamber and measuring the time they remained motionless, or frozen, presumably in fear of receiving more footshocks.

To block CREB function, the scientists developed transgenic mice that had an unusual response to the drug tamoxifen. In these animals, the drug prevented CREB from binding to DNA, and so blocked CREB activation of memory-associated genes.

When tamoxifen was administered before fear training, the duration of freezing time was unaffected 2 hours after training but was considerably reduced when measured 24 hours after training. This suggested that CREB-associated genes do not play a role in the creation of short-term memories, which are temporary memories that last for seconds or hours. However, they apparently do play a role in forming long-term memories, which are formed after about a day and stay in the brain for perhaps the rest of the animal's life.

A subsequent experiment showed that CREB was important not only for the initial consolidation of long-term memories but also for a recently discovered later stage in which reactivation of a memory makes it temporarily unstable. While the memory is in this transient plastic state, the brain seems to decide whether the memory should be retained, and if so, how firmly entrenched in the brain it should be.

In this experiment, the transgenic mice were not given fear training before tamoxifen but were given tamoxifen before placing them again in the footshock chamber, although this time without giving them footshocks. Results showed that disrupting CREB function during this second visit to the chamber, which reactivated

the memory of footshocks, reduced the freezing times measured 24 hours later. This indicated that blocking CREB function during memory reactivation at least partially disrupted the permanent storage of that memory.

"This editing phase gives the brain a chance to decide whether or not to keep a new memory permanently, because the memory may no longer be useful or may even be incorrect," explains Dr. Silva. In the case of the footshock memory, blocking CREB during the editing stage did not eliminate the memory, only weakened it, as shown by the fact that the tamoxifen-treated mice still froze when placed back in the footshock chamber, although for shorter periods of time. "Once highly emotional memories are laid down in the brain, they are very hard to get rid of," notes Dr. Silva. This is as it should be, he says, because highly emotional memories usually give rise to concepts that are important to the survival of the animal, such as avoiding places where predators are likely to be encountered. According to Dr. Silva, initial creation of intense memories probably involves high CREB levels to assure that the memories are learned quickly and remembered well.

These studies of CREB and memory are supported in part by two NCRR-DRI programs. One program, called Institutional Development Awards (IDeA), provides funds for developing the research infrastructure at institutions, such as the University of Puerto Rico, that are located in states that historically have not received significant amounts of competitive NIH funding. A component of the IDeA Program, called Centers of Biomedical Research Excellence (COBRE), enables these institutions to develop a multidisciplinary research center with a thematic science focus. For example, the University of Puerto Rico used COBRE funds to establish the Center for Molecular, Developmental, and Behavioral Neuroscience, where Dr. Peña is a researcher.

The other NCRR-DRI program is the Specialized Neuroscience Research Program (SNRP), also funded by the National Institute of Neurological Disorders and Stroke and the National Center on Minority Health and Health Disparities. SNRP grants are given to minority institutions to help them further the careers of junior faculty neuroscientists, such as Dr. Peña. To this end, the program requires that these young investigators team up with established neuroscientists, such as Dr. Silva.

According to Dr. Peña, the SNRP award has provided several benefits. "SNRP funds have enabled us to purchase the specialized equipment needed to perform this type of research," she says. "Also, the interaction between our lab and Dr. Silva's lab has helped create an environment that stimulates ideas and research."

Dr. Sandra Peña de Ortiz (left), with the help of lab technician Ivan Santos, identifies the neural genes activated by CREB during fear conditioning in mice. *(Photo by J. Perez-Mesa, University of Puerto Rico, Rio Piedras)*

The collaboration has benefited both research teams, says Dr. Silva. "Our lab is focused on the molecular and cellular events that turn on CREB and the behavioral consequences of these processes, whereas Dr. Peña's lab is more interested in the molecular processes downstream of CREB," he says. "It's been a perfectly complementary relationship."

—*Steven Stocker*

This research is supported in part by the Division of Research Infrastructure of the National Center for Research Resources, the National Institute of Neurological Disorders and Stroke, the National Center on Minority Health and Health Disparities, the National Alliance for Research on Schizophrenia and Depression, and the McKnight Endowment Fund for Neuroscience.

For more information about the NCRR Division of Research Infrastructure, see www.ncrr.nih.gov/research_infra.asp.

Additional Reading

1. Kida, S., Josselyn, S. A., Peña de Ortiz, S., et al., CREB is required for the stability of new and reactivated fear memories. *Nature Neuroscience* 5:348-355, 2002.

The Quest for the Perfect Pig Organ

Nearly 80,000 people are on the waiting list for organ transplants and about 6,000 die each year while waiting, according to the United Network for Organ Sharing. Many scientists consider pig organs to be a promising substitute for human organs, although cross-species transplantation, or xenotransplantation, often leads to organ rejection. NCRR-supported investigators recently have taken a major step toward overcoming this obstacle by producing knockout pigs in which a rejection-inducing gene is disabled.

A major problem with transplanting pig organs into humans is that the surfaces of pig cells are studded with sugar-based molecules called alpha-1,3-galactosylated moieties, which humans and many other primates lack. "When a pig organ is transplanted into a primate, this sugar is the first thing that is recognized as foreign, and it triggers the immune system to reject the organ," says Dr. Randall S. Prather, professor of animal science at the University of Missouri-Columbia.

Dr. Prather and his collaborators at Immerge BioTherapeutics in Charlestown, Massachusetts, have been attempting to knock out both copies of the pig gene that produces the enzyme alpha-1,3-galactosyltransferase (GGTA1), which helps transfer the sugar molecules onto the surface of pig cells. Like most genes in animal cells, GGTA1 genes occur in pairs, with one copy inherited from the father and one from the mother. "When GGTA1 gene expression is completely knocked out, the sugar should not be displayed on the cell surface, and thus the organs should not be immediately rejected," Dr. Prather says.

The first step toward the ultimate goal of producing pigs that totally lack GGTA1 is to knock out one copy of the gene. To accomplish this, scientists at Immerge first replaced a GGTA1 gene in fetal pig cells with an inactive version of the gene. The cells were then frozen and shipped to Dr. Prather's laboratory, where the cells' nuclei were removed and inserted into pig egg cells that lacked nuclei. The resulting genetically modified embryos were placed into surrogate mothers, ultimately yielding seven piglets—born in September and October 2001—that each lacked one copy of the GGTA1 gene. Three of the animals died shortly after birth, but four are still alive and have remained healthy.

This study marks the first success at knocking out a pig gene, although scientists have previously added genes to pigs. For instance, last year Dr. Prather led a research team that created a line of "glowing" pigs, to which was added the jellyfish gene for green fluorescent protein. This transgenic research, funded in part by NCRR, paved the way for the knockout studies.

The absence of one GGTA1 gene does not seem to unduly affect the pigs, who appear normal and healthy, says Dr. Julia Greenstein, an immunologist and the CEO of Immerge. Her company received Small Business Innovation Research Grants from NCRR to fund this knockout research. Some of the knockout pigs exhibited abnormalities—such as tendon deformities and heart defects—that have been seen in other cloned animals. However, these conditions appeared unrelated to the knocked-out gene, says Dr. Greenstein. She notes that knockout mice lacking both copies of the GGTA1 gene are normal and healthy, which makes her optimistic that double knockouts of the same gene in pigs will not

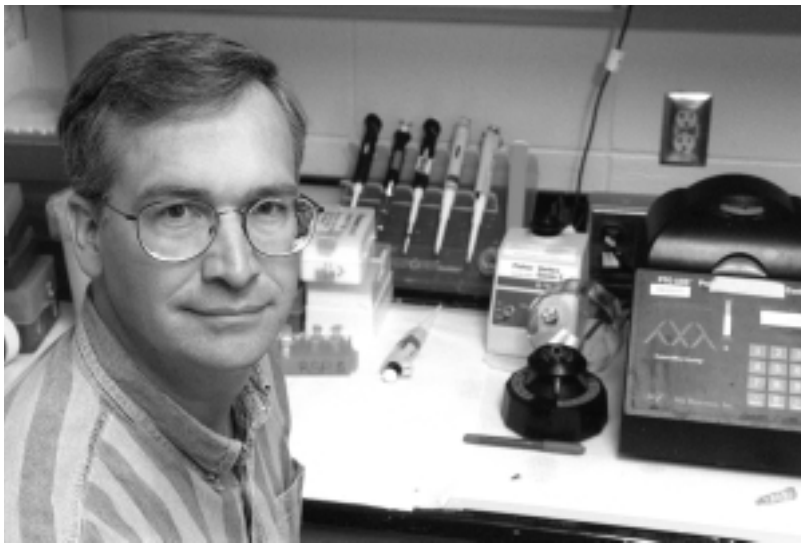
This study marks the first success at knocking out a pig gene.

These miniature pigs, shown here shortly after birth, have been genetically modified to make their organs more acceptable to human immune systems. With further genetic modification, organs from this pig strain might be transplanted into humans. *(Photo by Jim Curley, University of Missouri Office of Extension and Agricultural Information)*

cause severe abnormalities or make the organs unsuitable for transplantation.

Dr. Prather and his colleagues at Immerge used a strain of miniature pigs developed 30 years ago at the National Institutes of Health. These miniature pigs offer several advantages over the larger domestic pig, which was recently used by another research team to also create animals with a single GGTA1 gene knocked out. One potential benefit of miniature pig organs is that they are closer in size to human organs than are those of the domestic pig.

In addition, the line of miniature pigs used by Immerge appears to be incapable of transferring pig-specific viruses, called porcine endogenous retroviruses (PERVs), to human cells. This has been demonstrated in vitro, although the potential of in vivo infection has not been tested. PERVs are carried by all pigs, and the consequences of passing them on to humans are unknown.



Dr. Randall Prather produced the world's first knockout pigs, which lack one copy of a specific gene. *(Photo by Jim Curley, University of Missouri Office of Extension and Agricultural Information)*

The next step in creating transplantable pig organs is to knock out both genes for GGTA1. The Immerge research team is pursuing two approaches toward this goal. One involves genetically engineering pigs that completely lack functional versions of GGTA1, and the other involves inbreeding pigs that lack one copy of the gene in order to produce offspring that lack both. Dr. Greenstein predicts that at least one of these approaches will yield double-knockout pigs within a year and a half.

But even if the researchers do knock out both copies of the gene, rejection of pig organs may still occur, says Dr. Greenstein. To "re-educate" a patient's immune system and ensure tolerance to transplanted pig organs, Immerge is experimenting with concurrent transplantation of pig organs along with pig tissues, such as the thymus or bone marrow, that generate immune cells. "This approach works in small animal models, but nobody has tried it yet in pig-to-human transplantation," she says.

"As soon as we accomplish the double knockout, we'll try transplanting pig organs into baboons," says Dr. Greenstein. These studies could begin within two years. Baboons are an appropriate model because, like humans, they lack the GGTA1 gene and produce the same kind of antibodies to alpha-1,3-galactosylated moieties. Follow-up of six months to a year would be necessary to ensure that the baboons are not infected with PERVs and that the transplanted organs are functioning properly. At that point, clinical trials in humans could begin. "The heart and kidneys are the first organs that might be tried clinically," says Dr. Greenstein. Other possibilities include transplantation of islet cells from the pig pancreas for the treatment of type 1 diabetes.

Dr. Prather notes that the pig knockout technique may have even broader applications beyond xenotransplantation. For instance, knocking out specific genes may enhance meat production or enable creation of pig models of human diseases.

—*Steve Mitchell*

This research is supported by the Division of Comparative Medicine of the National Center for Research Resources and by the University of Missouri-Columbia.

For more information about the NCRR Division of Comparative Medicine, see www.ncrr.nih.gov/comparative_med.asp.

Additional Reading

1. Lai, L., Kolber-Simonds, D., Park, K. W., et al., Production of α-1,3-galactosyltransferase knockout pigs by nuclear transfer cloning. *Science* 295:1089-1092, 2002.
2. Oldmixon, B. A., Wood, J. C., Ericsson, T. A., et al., Porcine endogenous retrovirus transmission characteristics of an inbred herd of miniature swine. *Journal of Virology* 76:3045-3048, 2002.
3. Park, K. W., Cheong, H. T., Lai, L., et al., Production of nuclear transfer-derived swine that express the enhanced green fluorescent protein. *Animal Biotechnology* 12:173-81, 2001.

Critical Resources

NIH Expands National Gene Vector Laboratory Services

By inserting therapeutic genes directly into target cells, gene therapy offers a promising strategy for treating genetic defects and other diseases at their molecular source. But for the field to reach its full potential, scientists must have access to specialized research resources, like those offered by the National Gene Vector Laboratories (NGVLs). Since it was established in 1995, the NGVL network has helped to advance the field of gene therapy by supporting state-of-the-art laboratories that produce clinical-grade gene vectors—the "molecular taxis" that carry therapeutic genes into target cells. "These vectors are offered to qualified clinical investigators at no cost whatsoever to them," says Dr. Richard Knazek, the NGVL program officer in NCRR's Division of Clinical Research.

Now NCRR, in collaboration with eight other NIH institutes, hopes to enhance gene therapy research even further by expanding both the services and the number of sites within the NGVL network. In addition to three vector-production facilities, the network now includes two laboratories that perform preclinical toxicology testing of vectors, a frequent prerequisite for human studies. The coordinating center for all five NGVL sites is located at Indiana University in Indianapolis. These nationally shared resources are expected to help overcome the many obstacles scientists face when moving their gene therapy studies from the laboratory to the clinic.

Dr. Kenneth Cornetta heads the Coordinating Center of the National Gene Vector Laboratory program. *(Photo by Tex McCormick, Indiana University)*

For preliminary work in cell culture and animals, researchers often can obtain adequate quantities of vectors from their own institutions. But for clinical trials, vectors must be made in larger quantities and manufactured under strict quality guidelines. These steps require production facilities that most institutions cannot afford to build and operate.

"There's a complex series of tests to ensure that no contaminants have entered the production process," says Dr. Kenneth Cornetta, professor of medicine at Indiana University and director of the university's NGVL. All of these procedures—known as "good-manufacturing practices" required by the U.S. Food and Drug Administration—are followed by the three NGVL vector production facilities, each of which specializes in different types of gene vector.

The NGVL at Indiana University—Dr. Cornetta's laboratory—produces retroviral vectors; the NGVL at Baylor College of Medicine in Houston, Texas, manufactures adenoviral vectors; and the NGVL at City of Hope National Medical Center and Beckman Research Institute in Duarte, California, specializes in plasmid DNA (or nonviral) vectors. "People often ask which of these gene vector systems is superior," says Dr. Cornetta, "but each one has its advantages and disadvantages."

Retroviral vectors were one of the earliest vector types developed for gene therapy. One current study uses a retroviral vector to transfer a multidrug resistance (MDR) gene into hematopoietic, or blood-forming, stem cells to protect them from certain anticancer drugs. These drugs, which kill tumor cells, also have the unfortunate side effect of killing these stem cells, thereby depleting the patients of blood cells. To transfer the gene, the hematopoietic stem cells are harvested from peripheral blood, genetically altered, and then injected back into the patient's blood. "In Phase I trials, the MDR gene persisted for as long as one year," says Dr. Cornetta. However, the major limitation to retrovirus is that it's not very good at infecting nondividing cells—and stem cells generally do not divide."

An alternative type of retroviral vector, based on the lentivirus, appears better able to infect nondividing

cells and integrate well into the host cell's genome. Lentiviral vectors are now under development at Indiana University and elsewhere, as are vectors based on the herpes simplex virus, which targets neural tissue.

Like lentivirus, adeno-associated virus (AAV) can infect nondividing cells and integrate into the target cell's genome, but less efficiently. However, those genes that are successfully delivered via AAV appear to persist long term, observes Dr. Malcolm Brenner, director of the Center for Cell and Gene Therapy at Baylor College of Medicine.

When injected into the tumor, the vector transfers a "suicide gene" into cancerous cells.

The Baylor NGVL specializes in production of adenoviral vectors, which have the disadvantage of inducing a measurable immune response, but the potential advantage of limited integration into the host cell's genome. "Adenoviral vectors are very good if you want high-level, relatively short-term expression of therapeutic genes," says Dr. Brenner. "Because their effects are transient, advenoviral vectors are appropriate for treating cancer cells but not for correcting genetic defects."

At Texas Children's Hospital in Houston, adenoviral vectors produced at Baylor are being used in an experimental treatment for children with retinoblastoma, a cancer of the eye that traditionally requires surgery. When injected into the tumor, the vector transfers a "suicide gene" into cancerous cells. When affected eyes are treated with the drug gancyclovir, tumor cells that express the gene are destroyed.

Because cells that have been genetically transformed using viral vectors often lose their ability to express the transferred gene, researchers are investigating other agents for transferring genes. The City of Hope NGVL is producing DNA plasmids, or "naked DNA," circular pieces of bacterial DNA into which a therapeutic gene has been spliced. These vectors have the advantage of producing little or no immune response.

Two additional NGVL centers—located at the University of Florida in Gainesville and at the Southern Research Institute in Birmingham, Alabama—add a significant new capability to the network: preclinical toxicology testing of gene vectors. "Toxicology studies have emerged as another hurdle for gene therapy researchers, because their costs can be as prohibitive as vector production," says Dr. Cornetta. The NGVL network will perform toxicology studies, including tests of blood chemistry and histopathology of multiple organs, free of charge for qualified investigators.

Unlike toxicology studies funded by private sources, which remain proprietary, the results of the NGVL toxicology studies will be accessible through the NGVL Web site, which also has information on applying for NGVL-assisted studies. The first toxicology database will focus on AAV.

Making toxicology data freely available through the Internet is important because toxicities are most often related to the vector itself rather than to the particular therapeutic gene it carries. "Different researchers using the same vector could benefit from seeing each other's toxicology data," says Dr. Terry Flotte, professor of pediatrics and director of the NGVL at the University of Florida. "These data could forewarn of potential toxicities, improve patient safety, and save researchers from unnecessarily repeating studies."

In a related line of research, the Florida NGVL is producing a reference AAV vector. Historically, laboratories have used different techniques for measuring vector quantity, or dose, which makes it difficult to compare toxicology data from different clinical trials. With a reference AAV standard, scientists from several clinical sites can pool their data and identify the doses at which side effects occur.

"In the long run," concludes NCRR's Dr. Knazek, "we expect that the shared resources and enhanced capabilities of the NGVL network will not only advance the field of gene therapy, but also reduce the costs and enhance the safety of doing these studies."

—William Oldendorf

Core support for the National Gene Vector Laboratories is provided by the Division of Clinical Research of the National Center for Research Resources. Costs for vector production and toxicology studies are assumed by the following components of the National Institutes of Health: National Cancer Institute, National Institute of Allergy and Infectious Diseases, National Institute of Arthritis and Musculoskeletal and Skin Diseases, National Institute of Child Health and Human Development, National Institute of Dental and Craniofacial Research, National Institute of Diabetes and Digestive and Kidney Diseases, National Institute of Neurological Disorders and Stroke, and the Office of Rare Diseases.

To learn more about NGVL resources, or to obtain an application/request form for gene vectors or toxicology support, visit the NGVL Web site at www.ngvl.org, or contact the NGVL Coordinating Center at 317-274-0448.

News from NCRR

McKusick and Pestka Receive National Medals

Two NCRR-supported researchers were among the recipients of the 2001 National Medals of Science and Technology, the highest honors given in the United States to scientists and technological innovators. President Bush bestowed the medals at a White House ceremony on June 12, 2002.

Dr. Victor A. McKusick (above left), professor of medical genetics at the Johns Hopkins University in Baltimore, was one of the 15 recipients of the National Medal of Science. Widely regarded as the "father of medical genetics," Dr. McKusick started off as a cardiologist in the late 1940s, when he began to track the inheritance of Marfan's syndrome, a connective tissue disorder involving cardiovascular abnormalities. This eventually led him to collect information on the inheritance of genetic disorders in general, which helped establish medical genetics as a new field of medicine. From the 1960s to the 1980s, Dr. McKusick used the university's NCRR-supported General Clinical Research Centers for studying patients with genetic disorders. (For more information on Dr. McKusick, see the *NCRR Reporter*, Nov/Dec 1997, p. 14.)

Another NCRR grantee—Dr. Sidney Pestka (above right), professor and chair of the department of molecular genetics and microbiology at the University of Medicine and Dentistry of New Jersey in Piscataway—was one of the five recipients of the National Medal of Technology. Dr. Pestka is best known for developing the first biotherapeutic agents—interferons. These immune system proteins are currently used for treating diseases ranging from hepatitis to hairy cell leukemia. Dr. Pestka also developed the technology for recombinant DNA cloning and a new technique for purifying proteins. Dr. Pestka recently received an NCRR Shared Instrumentation Grant to establish a DNA microarray facility, which will allow researchers to study gene expression in organisms ranging from bacteria to humans.

Hyde Appointed Director of Primate Center

Dr. Dallas Hyde has been named the new director of the California National Primate Research Center (NPRC). Located at the University of California, Davis, the California NPRC is one of eight NCRR-funded NPRCs, which provide researchers with access to nonhuman primates for use in biomedical and behavioral studies.

Dr. Hyde is an expert on the biology of lung diseases, particularly asthma and pulmonary fibrosis. In a recent study conducted at the California NPRC, Dr. Hyde and his colleagues showed for the first time that exposing young monkeys to ozone, a component of smog, causes a disease similar to childhood asthma in humans. In other NCRR-funded research, Dr. Hyde has examined the role of immune cells in repairing ozone-induced lung injury and helped develop a computer program that rapidly analyzes images of lung structures. Dr. Hyde is a professor of anatomy, physiology, and cell biology in the university's School of Veterinary Medicine and has been serving as interim director of the NPRC since 2000.

NCRR's Advisory Council Gains New Members

Five new members have been appointed to the National Advisory Research Resources Council, the body that advises NCRR on policies, programs, and grant applications. The new members are:

Dr. Randall E. Dalton, a physician and surgeon at the Metro Richmond Ear, Nose, and Throat Physician and Surgeon Inc.; and assistant clinical professor of otolaryngology at the Medical College of Virginia in Richmond. His areas of expertise include surgery of head and neck tumors and treatment of hearing and balance loss.

Dr. Mark H. Ellisman, a professor of neuroscience and director of the National Center for Microscopy and Imaging Research at the University of California, San Diego. Dr. Ellisman is a pioneer in the development of three-dimensional light and electron microscopy.

Dr. James G. Fox, director of the division of comparative medicine and a professor in the division of bioengineering and environmental health at Massachusetts Institute of Technology in Cambridge. He researches infectious diseases of the gastrointestinal tract and their capacity to cause cancer.

Dr. John E. Maupin, Jr., president of Meharry Medical College in Nashville and an expert in administration, management, dental surgery, and general dentistry.

Dr. Paul G. Ramsey, vice president for medical affairs and dean of the School of Medicine at the University of Washington in Seattle. His research has focused on the development of methods to assess the clinical competence of physicians.

NCRR's New Web Site

NCRR recently launched its new Web site, featuring a streamlined design and improved navigational aids to help biomedical investigators locate resource-related information. The site, www.ncrr.nih.gov, provides information about research funding opportunities, access to scientific resources, publications, and news and events.

For those accustomed to the previous NCRR Web site, which was organized by NCRR division, information on divisions of Biomedical Technology, Comparative Medicine, Clinical Research, and Research Infrastructure still may be accessed directly from the homepage by clicking on the division name.

NCRR Support Aided National Academy Members

Four new members elected to the National Academy of Sciences in May have depended on NCRR-supported resources for their research. Election to the Academy is considered one of the highest honors that can be accorded a U.S. scientist or engineer. The four scientists are:

Dr. Francis V. Chisari, head of the division of experimental pathology and director of the NCRR-supported General Clinical Research Center at the Scripps Research Institute in La Jolla, California. Supported by NCRR for nearly 25 years, Dr. Chisari's research team examines immune system function during hepatitis B virus infection.

Dr. Jennifer A. Doudna, a professor in the department of molecular biophysics and biochemistry at Yale University in New Haven, Connecticut. Dr. Doudna and her colleagues determine the structure of unusual RNA molecules, such as ribozymes, which catalyze chemical reactions much like enzymes. In this research, Dr. Doudna uses the NCRR-funded Macromolecular Diffraction Biotechnology Resource at the Cornell High Energy Synchrotron Source (MacCHESS) in Ithaca, New York.

Dr. Morris Goodman, a professor in the department of anatomy and cell biology at Wayne State University School of Medicine in Detroit. By comparing the DNA sequences of certain genes among primate groups, Dr. Goodman and his colleagues determine how these genes evolved, and thereby gain insight into the evolution of certain human genetic diseases. Dr. Goodman relies on the NCRR-supported Washington National Primate Research Center in Seattle for much of his research.

Dr. Rowena G. Matthews, a professor of biological chemistry at the University of Michigan in Ann Arbor. Dr. Matthews and her colleagues discovered how the B vitamin folate reduces blood levels of homocysteine. High homocysteine levels are associated with heart disease and birth defects. Her studies have relied in part on the NCRR-supported MacCHESS facility. (For more information, see the *NCRR Reporter* Fall, 1999, pp. 5-7.)

(continues on back cover)

(continued from page 15)

Meyer Named Office of Review Director

Dr. John Meyer has been selected as the director of NCRR's Office of Review, where he will direct and coordinate the initial scientific and technical review conducted at NCRR of applications for grants and contract research. The office identifies and selects qualified experts to serve on the three NCRR review committees—Initial Review Group; Scientific and Technical Review Board on Biomedical and Behavioral Research Facilities; and Special Emphasis Panel—which are managed by the office.

"This position is critical to NCRR. The thorough and fair review of applications submitted by investigators is the cornerstone of NCRR programs," says NCRR Director Dr. Judith Vaitukaitis. "Dr. Meyer is an extremely capable manager who will assure that our grant programs continue to support the nation's health goals."

Dr. Meyer has a long tenure at NIH, with 27 years of Federal service. He has served in NCRR's Office of Review for the past four years, most recently as the deputy director and before that as a scientific review administrator (SRA). Prior to joining NCRR, Dr. Meyer served as an SRA with the Division of Extramural Activities at the National Cancer Institute; executive secretary with the Division of Extramural Activities at the National Institute of Allergy and Infectious Diseases; executive secretary with the Division of Research Grants at NIH; and research scientist at the National Institute of Dental Research. Before joining NIH, Meyer was a consultant with the Mayo Clinic and Mayo Foundation and assistant professor at the University of Minnesota.

American Chemical Society Honors Chait

The American Chemical Society presented the 2002 Frank H. Field and Joe L. Franklin Award for Outstanding Achievement in Mass Spectrometry to Dr. Brian T. Chait, director of the NCRR-funded National Resource for Mass Spectrometric Analysis of Biological Macromolecules at Rockefeller University in New York City. Using mass spectrometry, Dr. Chait has helped to determine the structures of the cell's potassium and chloride channels (see "NCRR Reports" on page 3) and the prion protein, which is associated with conditions such as "mad cow disease."

U.S. DEPARTMENT OF
HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health
National Center for Research Resources
One Rockledge Centre
6705 Rockledge Drive MSC 7965
Bethesda, MD 20892-7965

OFFICIAL BUSINESS
Penalty for Private Use, $300

Presorted Standard
U.S. Postage Paid
DHHS/NIH/RR
Permit No. G-818